SUPPLEMENTARY DATA

Inhibition of gastric tumor cell growth using seed-targeting LNA as specific, long-lasting microRNA inhibitors

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Content:

Table S1 Oligonucleotides used in this study

Oligonucleotide	Sequence (5' – 3')
Anti-miR-372 AS372	acGctCaaAtgTcgCagCacTtt
Anti-miR-373 AS373	acAccCcaAaaTcgAagCacTtc
Scrambled oligonucleotide SC372/373	caCgtAcaTagTgcAccGatAtt
Tiny anti-miR-372 TL372	CAGCACTTt
Tiny anti-miR-373 TL373	AAGCACTTc
Tiny anti-miR-17-5p TL17	AGCACTTTg
Tiny anti-miR-21 TL21	GATAAGCTa (*)
Tiny negative control (TLCo)	TCATACTAa (*)
Anti-miR-21	tcAacAtcAgtCtgAtaAgcTa
Anti-miR-17-5p	aCtaCCtGCaCtGtaaGCaCtttg
Anti-miR-93	ctAccTgcAcgAacAgcActTtg
U6	caCgaAttTgcGtgTcaTccTt
TDNA372	cagcacttt

LNA nucleotides are shown in upper case and DNA nucleotides in lowercase.

(*) according to Obad, S., dos Santos, C.O., Petri, A., Heidenblad, M., Broom, O., Ruse, C., Fu, C., Lindow, M., Stenvang, J., Straarup, E.M., et al. (2011) Silencing of microRNA families by seed-targeting tiny LNAs. *Nat. Genet.*, **43**, 371–378.

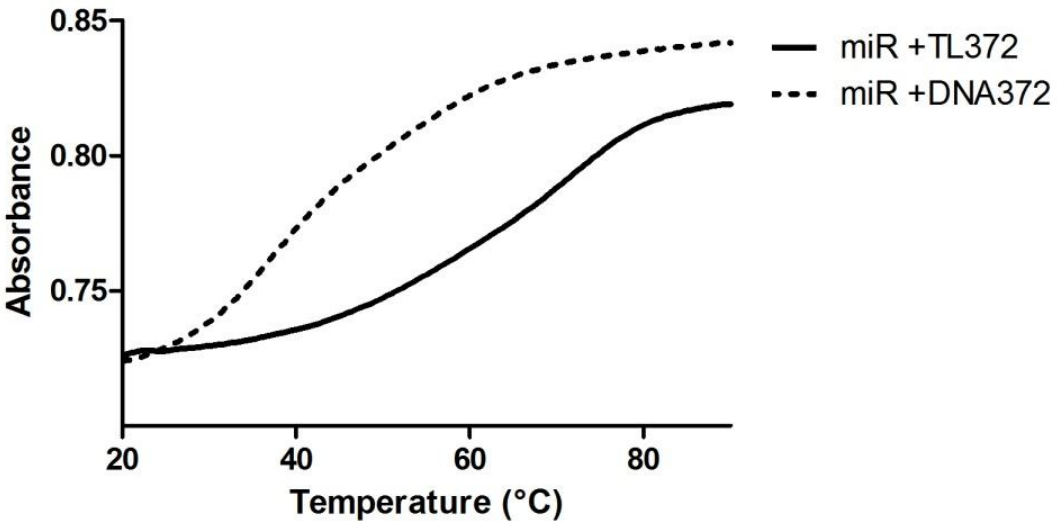

Figure S1: Determination of duplex melting temperature of the miR-372-3p in duplex with TL372 or TDNA372. The measures were performed with 2 μM of each component in 200 mM NaCl, 0,2 mM EDTA, 20 mM sodium cacodylate, pH 7.3, as indicated in Material and Methods. The Y-axis corresponds to the (A260nm-A310nm) curve of each duplex corrected with that of the buffer alone. A representative experiment out of 5 is shown.

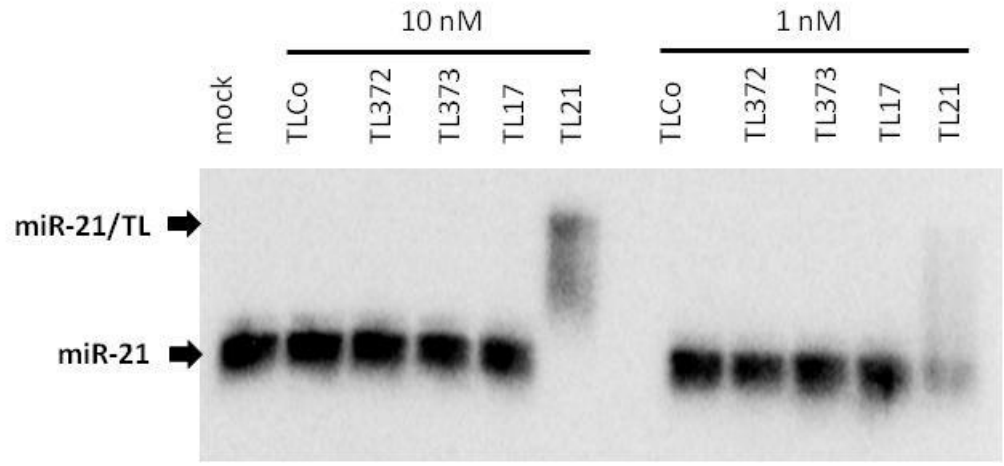

Figure S2: Non-denaturing northern blot analysis of miR-21 in AGS cells.

Cells were transfected with either TL372, TL373, TL17, TL21 or TLCo at 10 nM or 1 nM and grown for 5 days before RNA extraction and analysis.

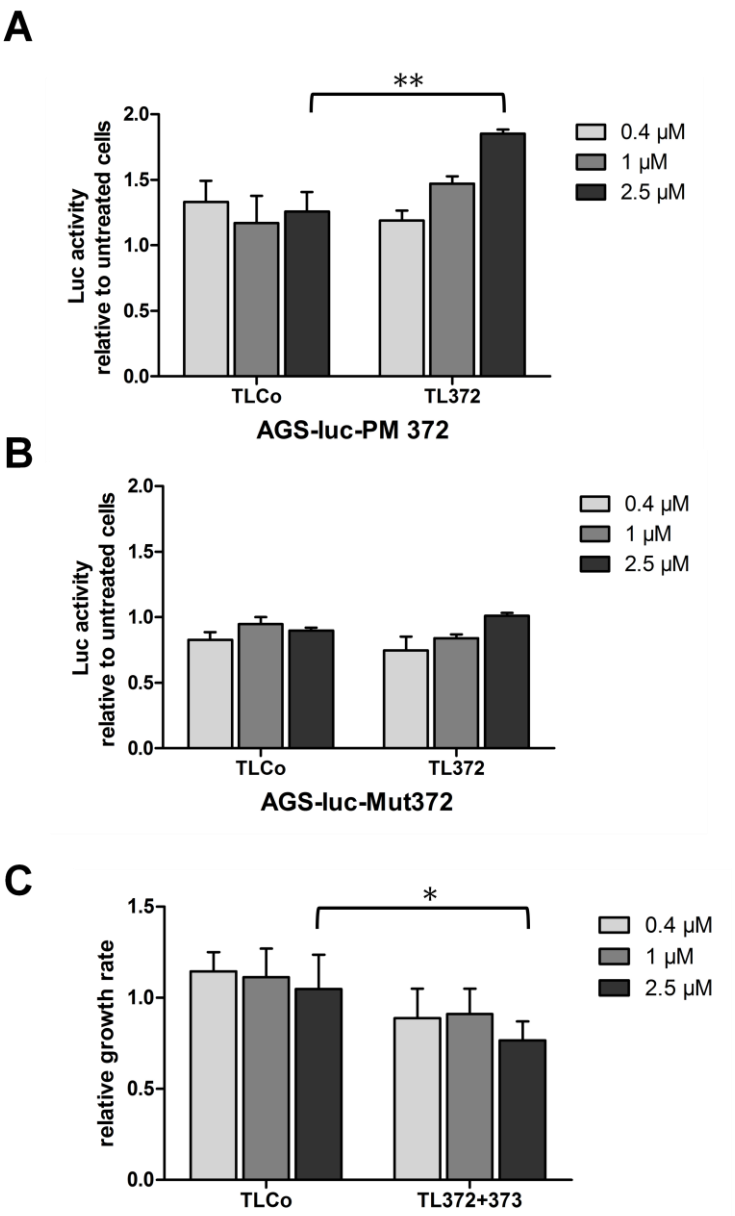

Figure S3. Unassisted 8-mer LNAs inhibit miR-372 and miR-373 functions.

(A,B) The stable reporter cell lines AGS-luc-PM372 (A) and AGS-luc-mut372 (B) were treated with increasing concentrations of TLCo or TL372 for 5 days. The bars represent the relative luciferase activities (mean ± SD, n = 6) of each reporter cell line normalized to total protein content and compared to untreated cells.

(C) Relative growth rate (mean ± SD, n = 6) of 8-mer LNA-treated cultured AGS cells between day 2 and day 5 post-treatment, compared to untreated cells.

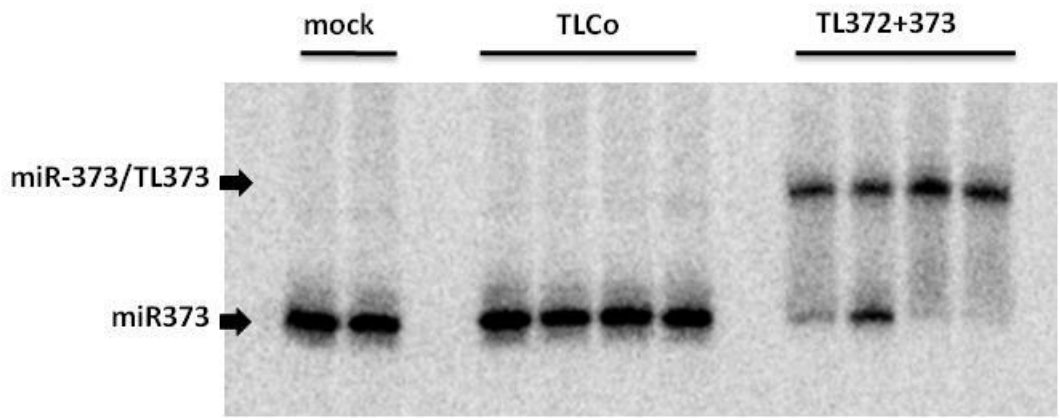

Figure S4: Non denaturing northern blot analysis of miR-373 in AGS-PM372 tumors treated with TL372+373 or TLCo at 5 nanomoles/mouse.

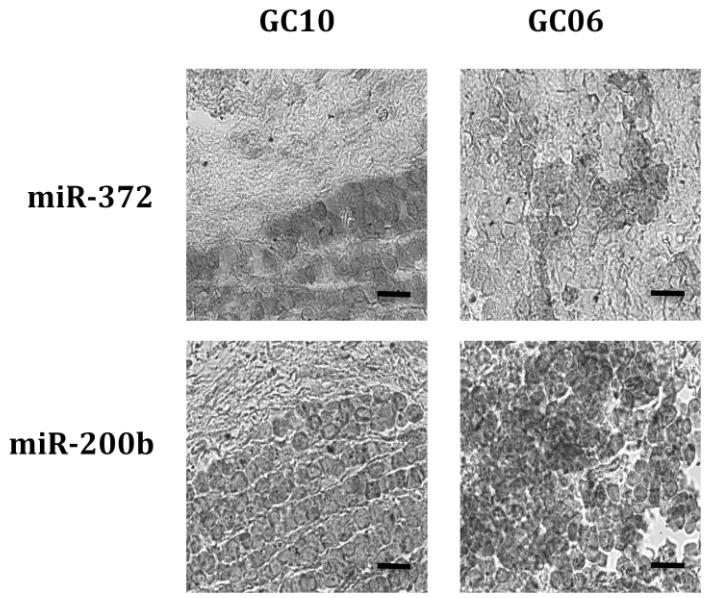

Figure S5: MiR-372 and miR-200b *in situ* hybridization.

Serial paraffin-embedded tissue sections of tumor xenografts of primary human gastric adenocarcinoma GC10 (lefts panels) and GC06 (right panels) in mice have been probed for miR-372 (upper pannels) or miR-200b (lower pannels). The positive miRNA expression appears in dark grey. Scale bars, 25 μm. Positively labeled cells appear in dark grey, whereas negative zones remain light grey like the negative controls (images not shown), in which the anti-DIG antibody has been omitted.

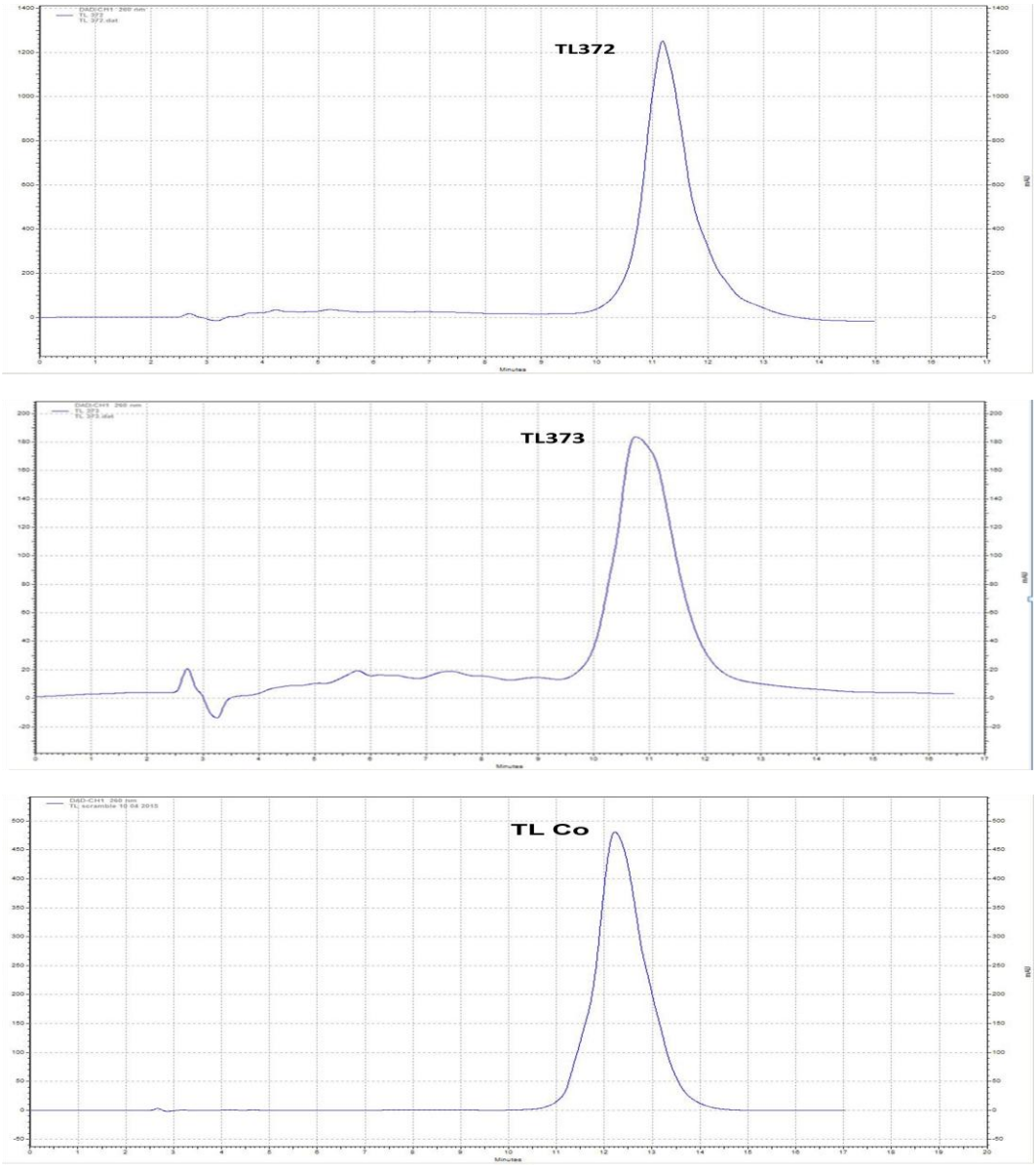

Figure S6 Chromatographs of TL372, TL373 and TLCo obtained by reverse phase, high pressure liquid chromatography on a Iachrom Elite Hitachi HPLC equipped with a C18 Isis column (5μm porosity, length/diameter = 250mm/4,6mm). The graphs represent the absorbance at 260nm as a function of the elution time (min). The elution conditions were the following: eluent A (v/v), 100% TEAA 0.1 M pH 7; eluent B, 20% TEAA 0.1 M pH 7/ 80% acetonitrile; starting with 97% A + 3% B, then 85%A + 15%B for 8 min, and back to initial conditions.